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The Development of Techniques and Strategies of Selection for Grain Yield, Protein and Kernel Hardness in Wheat Using Near-Infrared Spectroscopy

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FINAL REPORT FOR 1988/89 OF THE THIRD YEAR OF SASKATCHEWAN

AGRICULTURE DEVELOPMENT FUND RESEARCH GRANT

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The Development of Techniques and Strategies of Selection for Grain Yield, Protein and Kernel Hardness in Wheat Using Near-Infrared Spectroscopy.

This is the final report of the one year period of 1988/89. The emphasis of this report has been placed on the objectives outlined for this period. However, since this is also the final report of the three year research project, the most relevant findings from previous years are repeated herein. Furthermore, the yearly reports for the two preceding years are appended to this report (Appendices 1 & 2).

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ABSTRACT

Near-infrared spectroscopy has been successfully incorporated into the South Saskatchewan Wheat Breeding program headquartered at the Research Station, Agriculture Canada, Swift Current, to screen for the important quality characteristics of kernel protein in the CW Amber Durum and CW Hard Red Spring, and protein and kernel hardness in the Canadian Prairie Spring classes. The efficiency and productivity of this program have been significantly increased. Using this technique, breeders can now harvest test lines in Aug/Sept and determine kernel quality prior to sending material to a winter nursery for increase. Therefore, only those lines with desirable quality continue in the system. Although sample processing and instrument calibration are very important, NIR is considerably faster, less expensive and labour intensive than traditional chemical laboratory analyses.

Selection for grain yield and protein content simultaneously was effective. After three cycles of selection for yield and protein, lines from four different populations were developed which were higher than the mean of the checks for both grain yield and protein content. Selection strategies have been developed to select for kernel hardness and protein content and have been routinely applied in our wheat breeding program.

INTRODUCTION AND BACKGROUND

In order to remain competitive on the world wheat market, Canada must develop wheats which match the quality requirements of the importing countries. For example, high protein wheats tend to yield less grain; therefore, it is poor economics to use such wheats to compete for markets that do not require high levels of protein. Two of the most important quality components of wheat are protein and kernel hardness. However, traditional methods of measuring these components are expensive, labour intensive and slow. Near-infrared (NIR) spectroscopy represents an attractive alternative to the traditional methods as it is rapid and inexpensive. In 1977, R.D. Rosenthal presented a paper at the Amer. Assoc. of Cereal Chemists meetings, discussing the potential of using NIR to measure many agriculturally important constituents (e.g. protein, moisture, starch, etc.). Dr. P.C. Williams has successfully measured protein (1,2), moisture (1), and hardness (2) with NIR. However, NIR still remains more of an art than a routine technique and considerable study is required to ensure that it can be readily adapted by breeding programs. However, the advantages of integrating NIR into Canadian wheat breeding would be considerable.

Great variation exists in the genetic wheat stocks for most quality characteristics. Researchers such as Kushnir and Halloran (Australia) have demonstrated that desirable grain characteristics, such as protein content and kernel weight, can be transferred from ancestral wheats into adapted varieties.

OBJECTIVES

In general terms, the objective of this research was to increase the efficiency of wheat breeding programs by utilizing NIR technology to measure the quality components of protein and kernel hardness.

The three-year plan to achieve this objective was:

(a) Develop and evaluate calibration constants for a near infrared reflectance spectrophotometer suitable for measuring protein content and kernel hardness in wheat.

(b) Formulate selection strategies to select for grain yield and protein content in both Hard Red Spring and Amber Durum Wheat classes; and grain yield, protein content and kernel hardness in the Canadian Prairie Spring Wheat class (HY320 type).

(c) Implement evaluation and selection strategies that increase the efficiency of developing improved cultivars of Canada Prairie Spring, Hard Red Spring and Amber Durum Wheat classes.

Part of this objective has been realized previously as summarized in past reports. The specific objectives for the present review period were to:

a) Test the equations developed for protein and hardness on new sample sets representing increased genetic and environmental variability.

b) Summarize the results (for the three-year period) concerning the applicability and limitations of NIR for wheat breeding.

c) Incorporate routine NIR screening in a breeding program to improve efficiency.

EXPERIMENTAL METHODS:

(a) PLANT SAMPLES:

Canadian Western Amber Durum

(Previous report: Five Triticum diccocum lines which have high protein content but are unadapted to Saskatchewan, and low yielding, were hybridized previously with DT367, a high yielding line (20% higher than Wascana and Wakooma) but low in protein content. In 1985 the populations from these crosses were grown at Swift Current and Regina and 53 F_6 and F_8 lines were selected for further testing. During the winter of 1985-86, 265 head rows were grown at Brawley, Calif. to inbreed and multiply seed stocks. In 1986, 127 of the F_8 and F_{10} lines were grown at Swift Current and Regina. In 1987, 63 lines with improved protein content and good agronomic characteristics were grown in replicated tests at up to five locations. Seed samples were collected from each of these lines for each year and each location.)

To determine the genetic variation for grain yield and protein, and the relationship between these two traits, and to evaluate a strategy to select for both traits simultaneously, additional populations were studied. In 1988, 1924 breeding lines from 24 populations and in 1989, 2728 lines from 41 populations were grown in replicated tests at two locations. Grains samples were collected from both reps at one location. The ADF grant was used for sample preparation, analysis by NIR technology, and data analysis not only for CWAD samples but also for CPS and CWRS samples.

Canadian Prairie Spring

(Previous report: Thirty nine populations were produced previously to achieve the objective of developing high-yielding, sprouting resistant, white-seeded cultivars suitable for registering in this new wheat class. In 1985, 1695 hybrid lines, from these 39 populations, were grown at Swift Current and Regina. From these, 422 lines were selected with 5 head-rows being established for each selected line (422 x 5 = 2110) for multiplication and inbreeding in the Brawley winter nursery. In 1986, 920 lines were selected from the winter nursery and evaluated for performance relative to the CPS goal at Swift Current and Regina. In 1987, 1316 lines were evaluated for performance against the white CPS goal at two locations. Seed samples were collected from each of these lines grown at two locations for two years and also in a winter nursery.)

To determine the genetic variation for kernel hardness and protein content, and the relatinship between these two traits, and to evaluate a selection strategy to select for kernel hardness, additional populations were studied. In 1988, 2072 breeding lines from 17 populations were grown in replicated tests at two locations. Grain samples were collected from both reps at one location.

Canada Western Hard Red Spring

(Previous report: Three populations were produced previously to achieve the objective of developing a hard red spring wheat with elevated protein and/or grain yield. In 1986, 614 F_4 lines were evaluated in replicated tests at two locations. In 1987, 349 F_6 lines were progeny tested

for elevated yield and/or protein content at two locations. In 1987 an additional population consisting of 102 BC_1F_4 lines were handled in a similar manner.)

In 1988, 277 lines were progeny tested for elevated yield and/or protein content at two locations. In 1989 a sample of the better performing lines from each population were grown at three locations. As was done for the other classes of wheat, grain samples were collected from each of these lines for each location and in each year.

To determine the genetic variation for grain yield and protein, and the relationship between these two traits, and to evaluate a strategy to select for both traits simultaneously additional populations were studied. In 1988, eight populations and in 1989, nine populations were grown at two locations in replicated tests. Grain samples were collected from both reps at one location with the exception that for one population grain samples were taken from both locations.

For all populations in all years the data was summarized and experimental lines were selected on basis of yield, maturity, height, lodging, leaf and stem rust. An equal amount of grain was taken from each rep of the selected lines. Mean and standard deviation were calculated for both yield and protein as well as a regression of protein on grain yield.

In the 1987/88 report we indicated that a calibration set of Protein Standards were received from Dr. P.C. Williams of the Grain Research

Laboratory (GRL) in Winnipeg. During 1988/89 we obtained a second calibration set from Dr. Williams for kernel hardness (Kernel Hardness Standards).

(b) SAMPLE PROCESSING:

Although our sample processing methodology has not changed since our last report, the procedures are so important to ensure accuracy and reproducibility that they are repeated (and expanded upon) here.

Equilibration of grain:

An equilibration chamber is used to condition grain samples prior to grinding for NIR analyses. The chamber is maintained at 21°C and 60 % relative humidity. The samples are stored in envelopes with computer-generated identification labels on them; they are placed in this chamber for at least one week in order to adjust the moisture content of the grain to between 12 and 14%. The salient point is that all grain samples should be brought to the same moisture level as those used for instrument calibration. Other groups may find it more convenient to adjust the kernel moisture to values other than 12-14%; we chose this range as it is typical of grain at harvest and previous research done on such grain should be applicable.

Sample grinding:

Kernel hardness determines the particle size which results following grinding. The NIR measures kernel hardness indirectly by measuring particle size. All samples for this project were ground with a Udy Cyclone grinder (Udy Corp., Colorado) fitted with a constant feed adapter. If a constant feed adapter is not used, the particle size resulting from grinding, will reflect not only kernel hardness but also the speed at which the grain is delivered to the grinder. Only enough

samples were removed from the equilibration chamber as could be conveniently ground within a 2 hour period to prevent changes in grain moisture content prior to grinding. Immediately following grinding the samples were placed in metal containers and sealed to prevent moisture changes.

(c)NEAR-INFRARED:

The NIR instrument used was a Neotec 51A (Neotec, Silver Springs, MD). This instrument has six rotating filters in order to measure absorption at 768 wavelengths. Protein predictions were based on data recorded at wavelengths of 2142 and 2250 nanometers. For kernel hardness, a wavelength of approximately 1620 nanometer was used (2). The entire spectra of certain sample sets was also stored on computer.

(d) LAB ANALYSES:

Protein was measured at the Swift Current Research Station using the Kjeldahl procedure (3). Moisture contents were measured gravimetrically following 24 hours drying at 105°C. Kernel hardness for routine samples was measured at the Winnipeg Research Station; the method involved determination of grinding times (4).

(e) DATA ANALYSES:

All data were analyzed with SAS and SASGRAPH (SAS Institute,Cary,NC) running on a Microvax 2000 (Digital Equipment Corp.)

RESULTS AND DISCUSSION

(a) Development and Evaluation of Calibration Constants:

During the first year of this project we experienced serious reproducibility problems with our protein measurements. After considerable testing, discussions, and, unfortunately, time, we were able to identify two sources for the variation. Firstly, the NIR values varied due to fluctuations of the ambient room temperature. This problem was solved by transferring the instrument to a constant temperature room. Since the room temperature fluctuations were within the limits of the specifications, we assumed that our particular instrument was faulty. However, we have since learned that other research groups using instruments from different companies, have also found that temperature control is crucial to reproducibility. Therefore, we conclude that the problem may be of a general nature and should be considered in future applications.

The second source of variation proved to be our laboratory protein determination. It is of utmost importance to realize that the NIR is a 'black-box' technique; it is only as good as the methods used to calibrate it! We were able to determine that our laboratory protein determinations were not as accurate as we had previously believed. Furthermore, discussions with other research groups indicated that they had also found their laboratory methods less accurate than they previously believed.

The NIR instrument was supplied with an equation to predict protein in wheat based upon the near-infrared spectrum. The details of the this method are outlined in appendix 3. This equation was tested using the Protein Standards from GRL Winnipeg. We found that the results from the NIR correlated very highly with the true values for this set of samples (linear correlation coefficient > 0.95). However, a minor correction to the intercept

and a major correction to the slope of the Neotec equation was required to predict true protein values. Results obtained during this past year have indicated that a further refinement was required to correct for kernel hardness. Apparently the kernel hardness was interfering with the NIR protein measurement. We have found that slightly different intercept values are required for hard and soft wheats (Fig. 1). The equations that we are currently using are as follows:

% Protein (for soft wheats) = 31.42 + 7003.16 (Ratio)
% Protein (for hard wheats) = 32.06 + 7003.16 (Ratio)
- where Ratio is a valued determined from the NIR (see appendix 3)

We have also successfully incorporated sample processing methodology to predict kernel hardness on the NIR. The major requirement was found to be the equilibration of grain prior to grinding, as detailed above (Experimental Methods (c) sample processing). The wavelength of 1680 nanometers, previously used by Williams (2) was found to be completely adequate. Using the set of Kernel Hardness Standards we found a linear correlation between our NIR values and the Particle Size Index values (PSI the accepted laboratory measure of kernel hardness) greater than 0.96 (Fig. 2). The equation that we are currently using for kernel hardness is:

Kernel Hardness (PSI) = 132.787-460.932 (OD142)

- where OD142 is the OD value at PP 142 (1680 nanometers) (see appendix 3 for details)

(b) Strategies to Select for Grain Yield and Protein

The standard deviation is a measure of the variation for grain yield and protein concentration. In each population at all locations protein and yield varied (Table 1, 2, 3, and 4). Regression analysis assesses the relationship between two variables which may or may not be <u>causally</u> related. The regression coefficients (b) were small and often not significant. The

Figure 1. Predicted protein from near-infrared for hard and soft wheats.

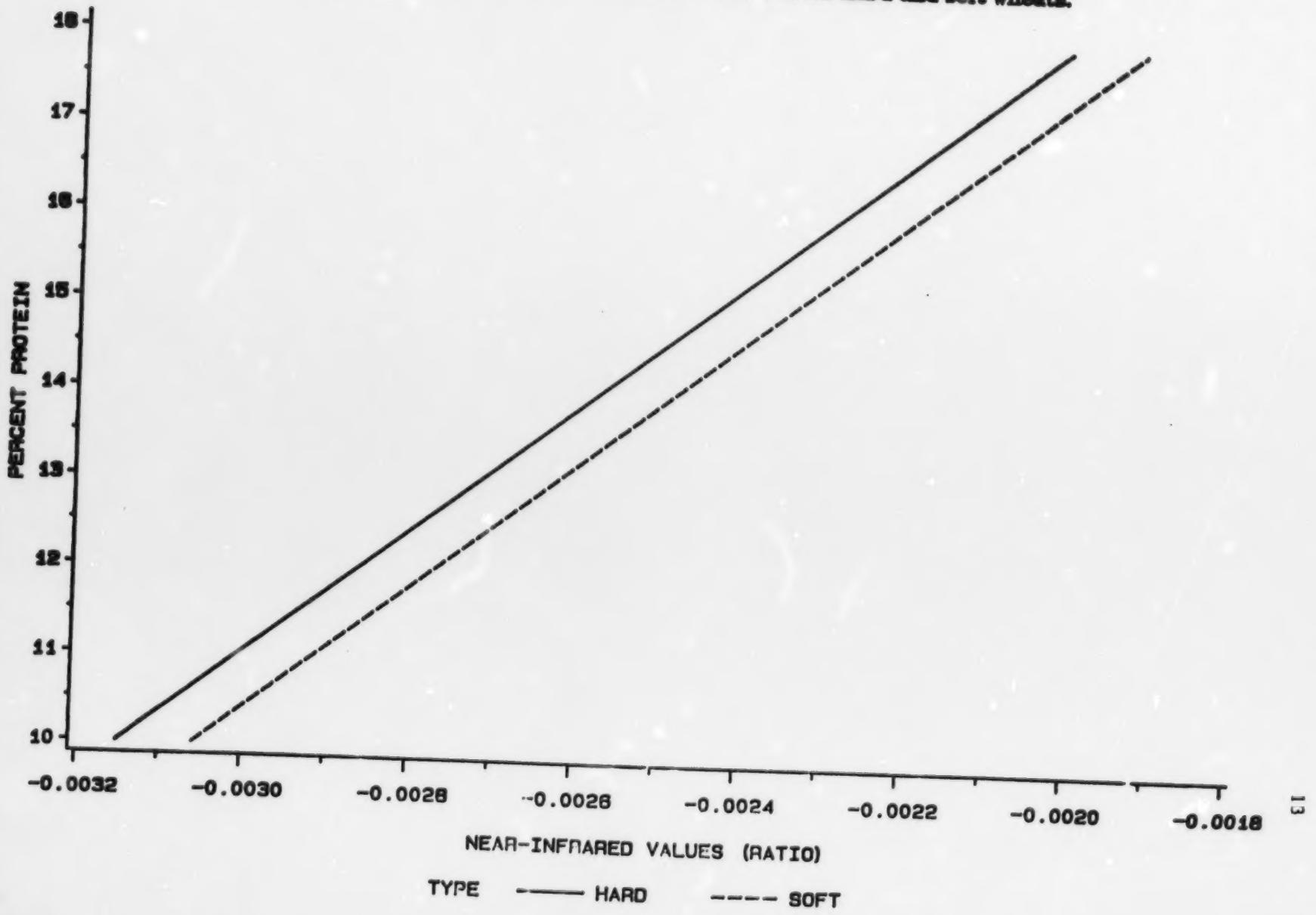
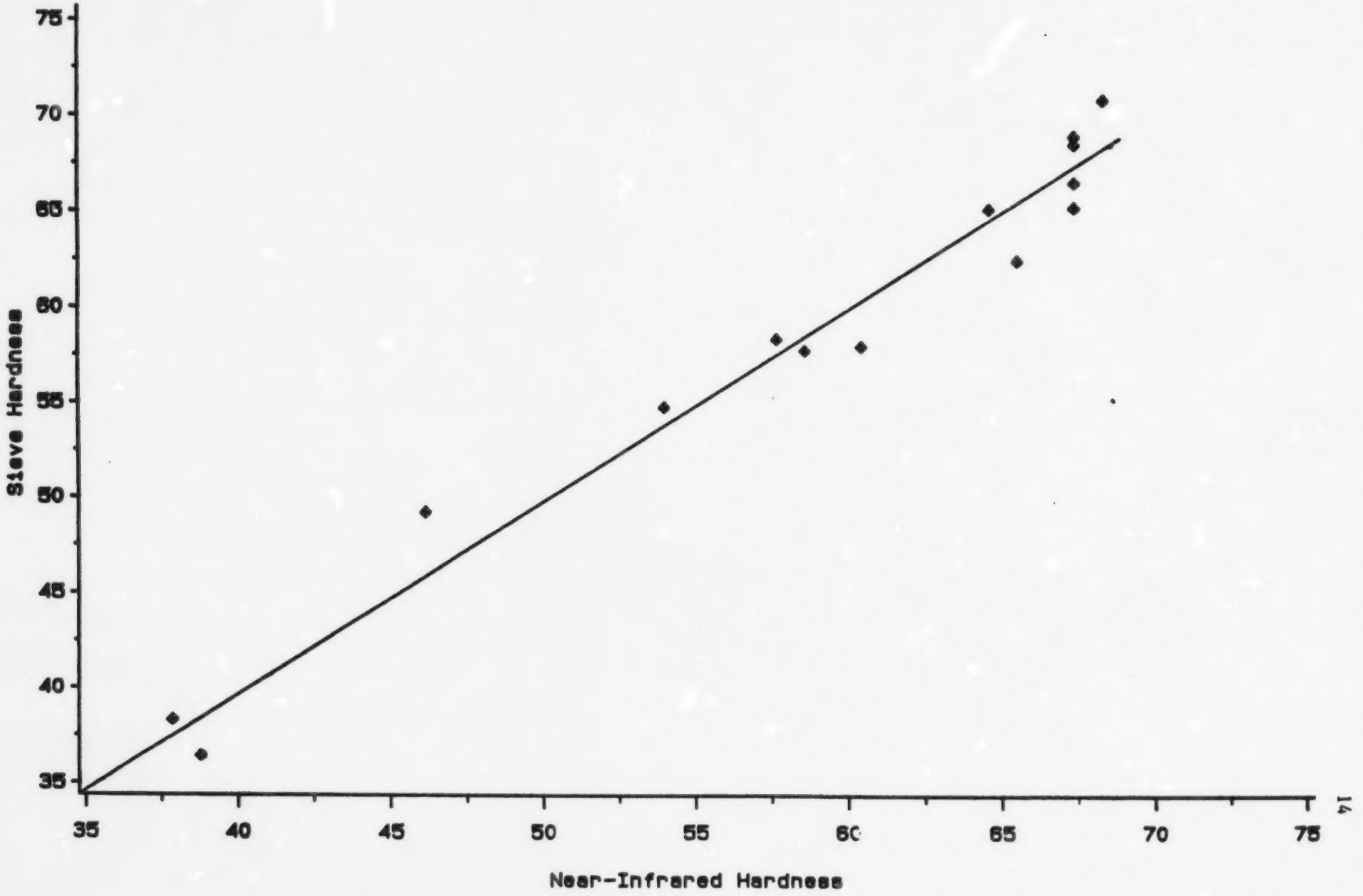


Figure 2. Kernel Hardness measured by Sieve and Near-Infrared Methods.



coefficients of determination (r^2) which is the proportion of the variance of the dependent variable (protein) that can be attributed to its linear regression on the independent variable (grain yield), were generally small (less than 0.10 i.e., less than 10% of the variation in protein could be attributed to variation in grain yield). This implied it should be possible to select for either increased yield, or protein, or both simultaneously.

Table 1. Regression of protein (NIR) on grain yield 1986

		n	b	r^2	Protein (%) x	Protein (%) SD	Yield (gms/plot) x	Yield (gms/plot) SD
8404	SC	99	-0.0038**	0.100	19.9	0.52	827	45.7
	Reg		-0.0011ns	0.076	17.8	0.55	1309	92.7
	Mean		-0.0020*	0.056	18.8	0.45	1068	54.7
8405	SC	86	-0.0039ns	0.032	17.8	0.99	813	45.1
	Reg		-0.0006ns	0.009	17.9	0.56	1191	87.6
	Mean		-0.0001ns	0.000	17.9	0.61	1002	50.1
8406	SC	55	-0.0014ns	0.009	18.5	0.58	772	40.5
	Reg		-0.0013ns	0.031	17.6	0.56	969	74.3
	Mean		-0.0009ns	0.007	18.1	0.47	871	42.6

n = selected portion of the population evaluated for both yield and protein.
b = regression coefficient.
r^2 = coefficient of determination.
x = mean.
SD = standard deviation.
*, ** = values are significantly different from zero P<= 0.05, and 0.01 respectively.
ns = values are not significantly different from zero P>=0.05.

Table 2. Regression of Protein (NIR) on grain yield 1987

		n	b	r^2	Protein (%) x	Protein (%) SD	Yield (gms/plot) x	Yield (gms/plot) SD
8404 + 8406	SC	48	-0.0027ns	0.059	21.0	0.38	775	43.7
	Reg		-0.0005ns	0.016	20.5	0.41	971	127.8
	Mean		-0.0013ns	0.038	20.7	0.36	873	73.8
8405	SC	58	-0.0036**	0.150	20.3	0.33	677	35.2

n = selected portion of the population evaluated for both yield and protein.
b = regression coefficient.
r^2 = coefficient of determination.
x = mean.
SD = standard deviation.
*, ** = values are significantly different from zero P<= 0.05, and 0.01 respectively.
ns = values are not significantly different from zero P>= 0.05.

Table 3. Regression of Protein (NIR) on grain yield 1988

		n	b	r^2	Protein (%) x	Protein (%) SD	Yield (gms/plot) x	Yield (gms/plot) SD
8404	SC	62	+0.0015ns	0.003	14.5	0.73	271	29.0
	IH		-0.0034*	0.074	13.9	0.60	573	47.9
	Mean		-0.0009ns	0.004	14.2	0.44	482	29.8
8405	SC	156	-0.0057*	0.034	13.5	0.77	278	25.1
	IH		-0.0017*	0.031	14.3	0.58	586	61.8
	Mean		-0.0042*	0.067	13.9	0.59	432	36.0
8406	SC	34	+0.0054*	0.171	14.4	0.46	274	35.9
	IH		-0.0070**	0.314	14.2	0.50	536	40.0
	Mean		-0.0026ns	0.064	14.3	0.34	405	32.6
L8509	SC	40	-0.0003ns	0.000	14.5	0.58	284	25.6
	IH		-0.0063ns	0.087	14.1	0.68	578	31.9
	Mean		-0.0057ns	0.072	14.3	0.47	431	29.9

n = selected portion of the population evaluated for both yield and protein.
b = regression coefficient.
r^2 = coefficient of determination.
x = mean.
SD = standard deviation.
*, ** = values are significantly different from zero P<= 0.05, and 0.01 respectively.
ns = values are not significantly different from zero P>= 0.05.

Table 4. Regression of Protein (NIR) on grain yield 1989 (8404, 8405, 8406, L8509).

	n	b	r^2	Protein (%) x	Protein (%) SD	Yield (gms/plot) x	Yield (gms/plot) SD
SC	90	-0.0021ns	0.031	15.1	0.47	845	38.5
IH		-0.0017*	0.052	16.5	0.53	873	59.4
Reg		-0.0016ns	0.031	16.6	0.45	672	57.3
Mean		-0.0025*	0.066	16.1	0.37	797	37.6

n = selected portion of the population evaluated for both yield and protein.
b = regression coefficient.
r^2 = coefficient of determination.
x = mean.
SD = standard deviation.
*, ** = values are significantly different from zero P<= 0.05, and 0.01 respectively.
ns = values are not significantly different from zero P>= 0.05.

The progeny tests of the populations 8404, 8405, 8406, and L8509 confirm that simultaneous selection for both traits was effective (Tables 5 and 6). In 1988 for each population averaged over two locations the mean yield was higher than that of the checks while the protein was equal to the checks (except L8509) or the yield was slightly lower but the protein was slightly higher (Table 5). In 1989 the selected lines were grown in 4 reps at 3 locations (Swift Current, Regina and Indian Head). The average of the lines for three of the populations was higher than the mean of the checks for both yield and protein (Table 6). The highest yielding line was 15% more than the mean of the checks at slightly higher protein. The highest protein line was 6.4% more than the mean of the checks and slightly higher in yield.

Table 5. Grain Yield and Protein of lines derived from four populations and compared to registered check cultivars.

	1988				1989			
	No of lines tested	Protein %	Yield %	Yield gms/plot	No of lines tested	Protein %	Yield %	Yield kg/ha
8404	62	14.2	102.7	422	17	16.1	106.5	2951
Mean of checks	8	14.2	100	411	4	15.8	100	2770
8405	141	13.9	106.7	434	46	16.1	103.2	2868
Mean of checks	15	13.9	100	407	4	15.8	100	2770
8406	34	14.3	98.5	405	5	15.8	103.2	2862
Mean of checks	8	14.2	100	411	4	15.8	100	2770
L8509	40	14.3	104.9	431	16	16.1	104.7	2900
Mean of checks	8	14.2	100	411	4	15.8	100	2770

Table 6. Grain yield and protein of selected lines and families deriving from populations 8404, 8405, 8406, and L8509 tested at three locations in 1989.

	No. of lines in family	Protein %	Yield % of x checks	Yield kg/ha
Highest Yielding line	1	15.91	115.2	3190
Highest Yielding Family	4	16.23	110.8	3068
Highest Protein Line	1	16.79	101.8	2820
Highest Protein Family	2	16.61	106.3	2945
Checks				
Columbus		16.42	96.4	2670
Katepwa		15.42	103.2	2860
Laura		15.96	99.6	2760
Neepawa		15.34	100.7	2790
Mean of Checks	4	15.78	100.0	2770

(c) Incorporation of Routine Screening for Protein by NIR in a Breeding Program

When other populations were studies, the relationship of protein and yield (measured by the regression coefficient 'b') was small, negative and frequently significant (Tables 7 and 8). This contrasts with the generally non-significant b-values for the first four populations studied (Table 1-4). However, the proportion of the variation associated with the relationship was generally quite small, indicating that it should be possible to select for both yield and protein in these populations also (Tables 7 and 8).

At Swift Current in 1988 the evapo-transpiration rate was very high while the growing precipitation was about normal which resulted in very low yields. While in 1989 evapo-transpiration was about normal and growing season precipitation was above normal which resulted in above average yields. The relationship between protein and yield was expressed in a similar manner in both years, indicating that genetic advance through selection

was possible under both conditions.

Table 7. Regression of protein (NIR) on grain yield - 1988.

Pop'n	n	b	r^2	Protein (%) x	Protein (%) SD	Yield gms/plot x	Yield gms/plot SD
8401-3AB	69	-0.0033ns	0.045	15.7	0.73	316	47.1
8401-8611	108	-0.0048ns	0.030	15.1	0.82	321	29.9
8501-2	93	-0.0055**	0.070	14.4	0.69	309	33.6
8503-4	122	-0.0122**	0.359	14.0	1.13	295	55.6
8508 SC	41	-0.0100**	0.349	15.0	0.59	275	34.7
IH	41	-0.0039ns	0.072	14.2	0.59	647	40.3
Mean	41	-0.0093**	0.196	14.6	0.54	461	25.7
8601	73	-0.0061**	0.176	13.5	0.69	321	48.1
8602	73	-0.0033ns	0.047	15.1	0.50	326	32.0
8611	28	-0.0017ns	0.008	15.9	0.38	262	20.9

n = selected portion of the population evaluated for both yield and protein.
b = regression coefficient.
r^2 = coefficient of determination.
x = mean.
SD = standard deviation.
*, ** = values are significantly different from zero P<= 0.05, and 0.01 respectively.
ns = values are not significantly different from zero P>= 0.05.

The genetic variation for yield and protein depends upon the parents used in the cross. For example in populations 8501 and 8502 there is variation for both yield and protein (Figure 3 and 4). But the possibility of deriving lines which are higher in yield, or protein or both yield and protein than the checks are better in population 8501 than 8502.

Table 8. Regression of Protein (NIR) on Grain yield - 1989.

Pop'n	n	b	r^2	Protein (%) x	Protein (%) SD	Yield gms/plot x	Yield gms/plot SD
8501, 3 SC	66	-0.0035ns	0.040	15.5	0.50	814	28.7
SV		-0.0046**	0.268	14.2	0.70	1064	61.3
Mean		-0.0045**	0.181	14.8	0.49	939	47.1
8502 SV	61	-0.0041**	0.184	14.6	0.49	933	50.5
8508, 8611	58	-0.0062**	0.158	14.6	0.95	770	60.7
8601, 2	51	-0.0051**	0.142	15.3	0.60	799	44.5
8701, 2	68	-0.0056**	0.136	14.7	0.71	862	46.3
8703	62	-0.0080**	0.295	16.5	0.57	787	38.6
8706	128	-0.0052**	0.088	14.6	0.62	754	35.2
8707	96	-0.0057**	0.118	14.6	0.74	750	45.1
L8900	58	-0.0020ns	0.018	14.7	0.50	803	33.9

n = selected portion of the population evaluated for both yield and protein.
b = regression coefficient.
r^2 = coefficient of determination.
x = mean.
SD = standard deviation.
*, ** = values are significantly different from zero P<= 0.05, and 0.01 respectively.
ns = values are not significantly different from zero P>= 0.05.

Evaluation of methods and development of strategies to select simultaneous for yield and protein in durum wheat followed similar procedures as that for hard red spring wheat data indicated that (1) the NIR technology can be used to measure protein in durum wheat (2) genetic variation for grain yield and protein exists (3) the relationship between yield and protein (b-value) was significant and negative, and (4) the proportion of the variation in protein that could be explained by its relationship to yield varied from 0.053 to 0.419 (Table 9 and Figures 5 and 6). The results are analogous to that for hard red spring wheat. Methods and strategies similar to that developed for the hard red spring wheats have been implemented to select for grain yield and protein content in durum wheat.

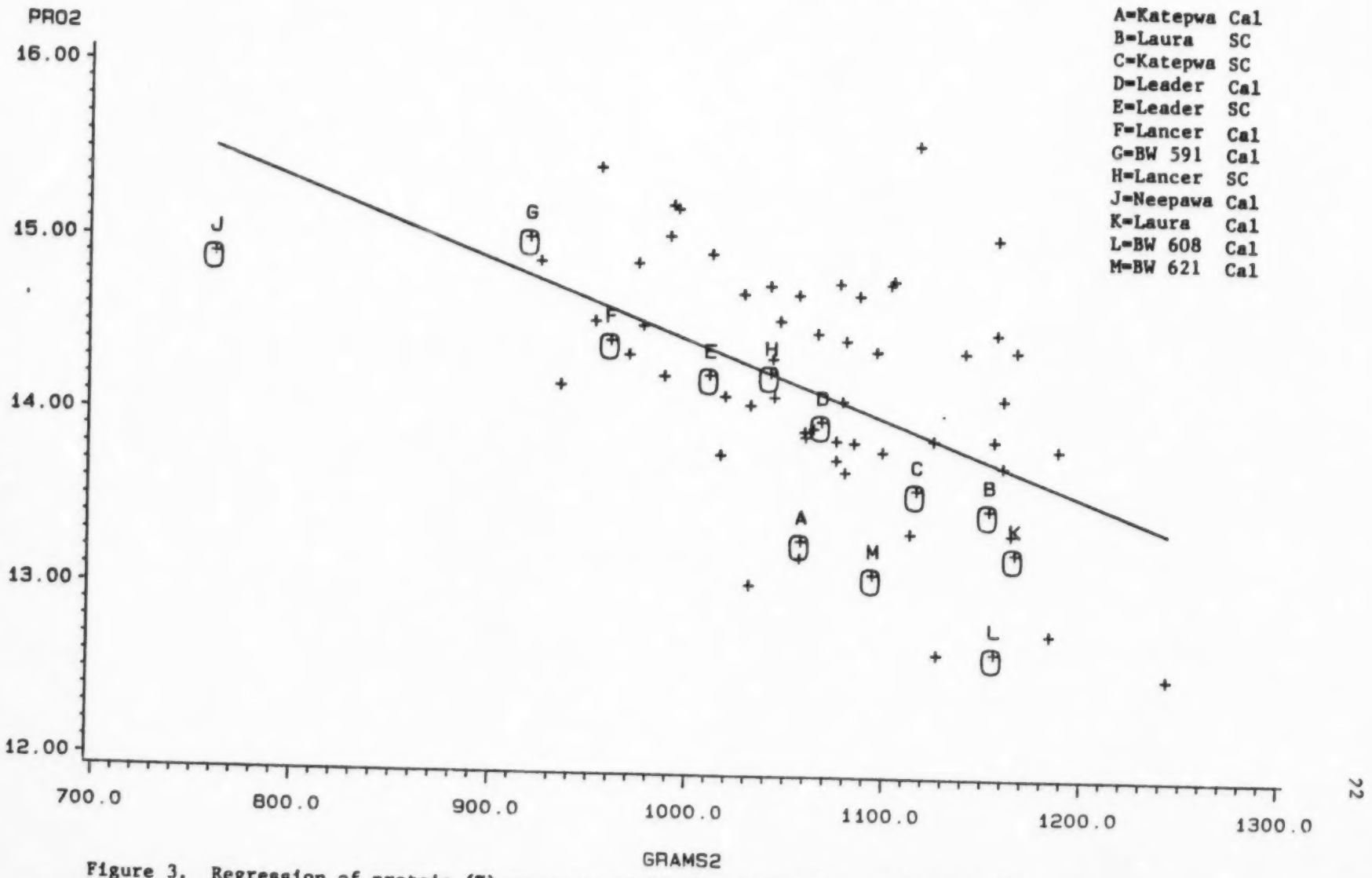


Figure 3. Regression of protein (%) on grain yield (gms/plot) (b= -0.0035ns, r^2= 0.040)

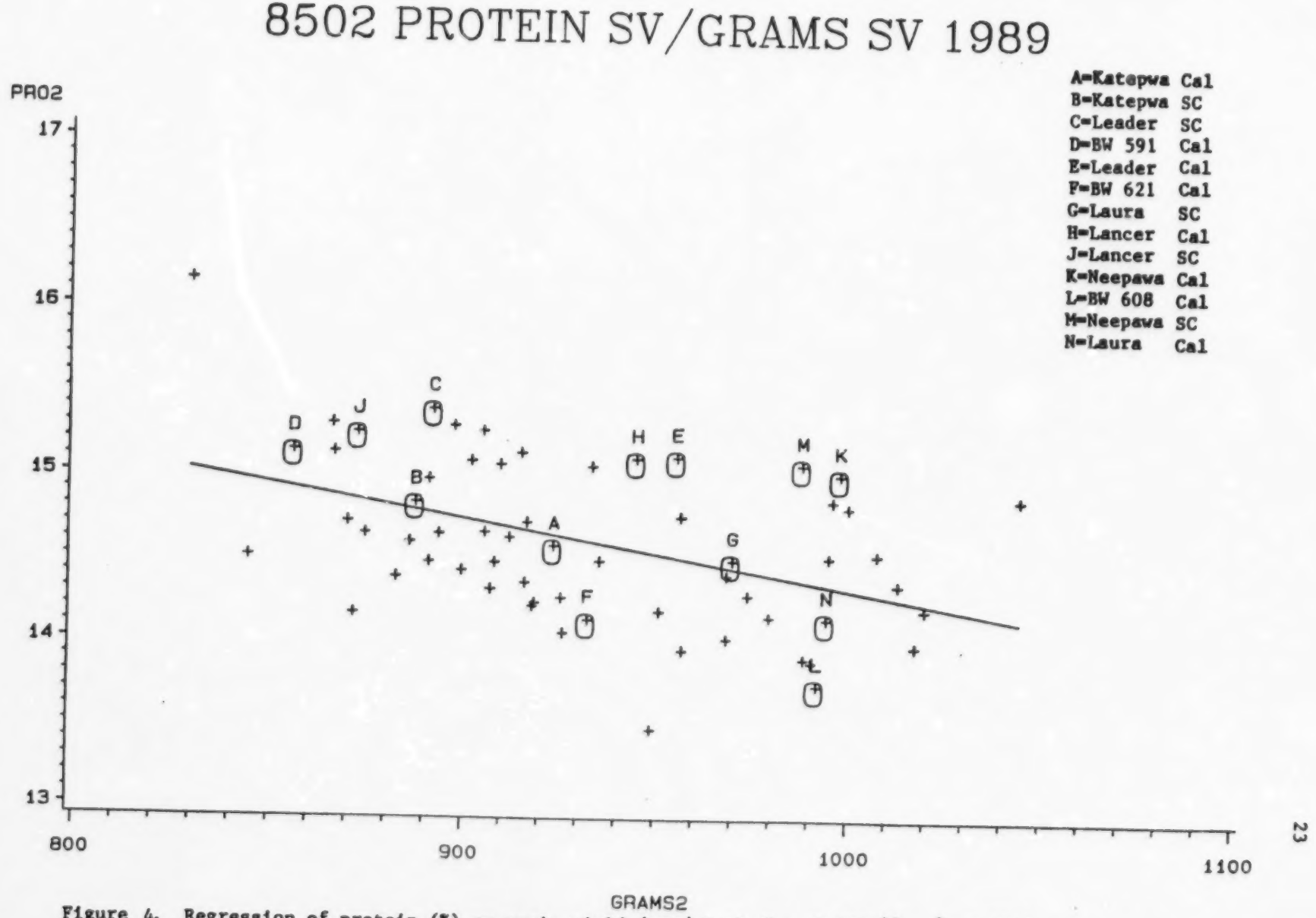


Figure 4. Regression of protein (%) on grain yield (gms/plot) (b= -0.0041**, r^2= 0.184).

Table 9. Regression of Protein (NIR) on grain yield - 1989

Population	n	b	r^2	Protein (%) x	Protein (%) SD	Yield (g/plot) x	Yield (g/plot) SD
8761A-2C	65	-0.0075**	0.254	14.4	1.01	795	67.1
8763A-4C	78	-0.0062*	0.070	14.9	1.01	730	43.3
8765A-9C	69	-0.0037**	0.104	18.1	0.85	655	72.9
8766A-8C+	211	-0.0085**	0.239	14.3	1.30	813	74.7
8770-71	47	-0.0102**	0.250	16.3	0.79	694	38.7
8772-73	76	-0.0075**	0.231	16.2	0.93	798	59.3
8774-75	84	-0.0099*	0.053	15.2	1.55	717	36.4
8661-84, 8778	86	-0.0083**	0.286	14.6	0.83	656	53.6
8663, 78, 83	91	-0.0058**	0.186	17.3	0.76	524	56.0
8674	32	-0.0096**	0.419	16.4	0.82	680	55.3
8670	54	-0.0031*	0.101	17.1	0.67	558	69.0

n = selected portion of the population evaluated for both yield and protein.
b = regression coefficient.
r^2 = coefficient of determination.
x = mean.
SD = standard deviation.
*, ** = values are significantly different from zero P<= 0.05, and 0.01 respectively.
ns = values are not significantly different from zero P>= 0.05.
+ = grain sample from one rep.

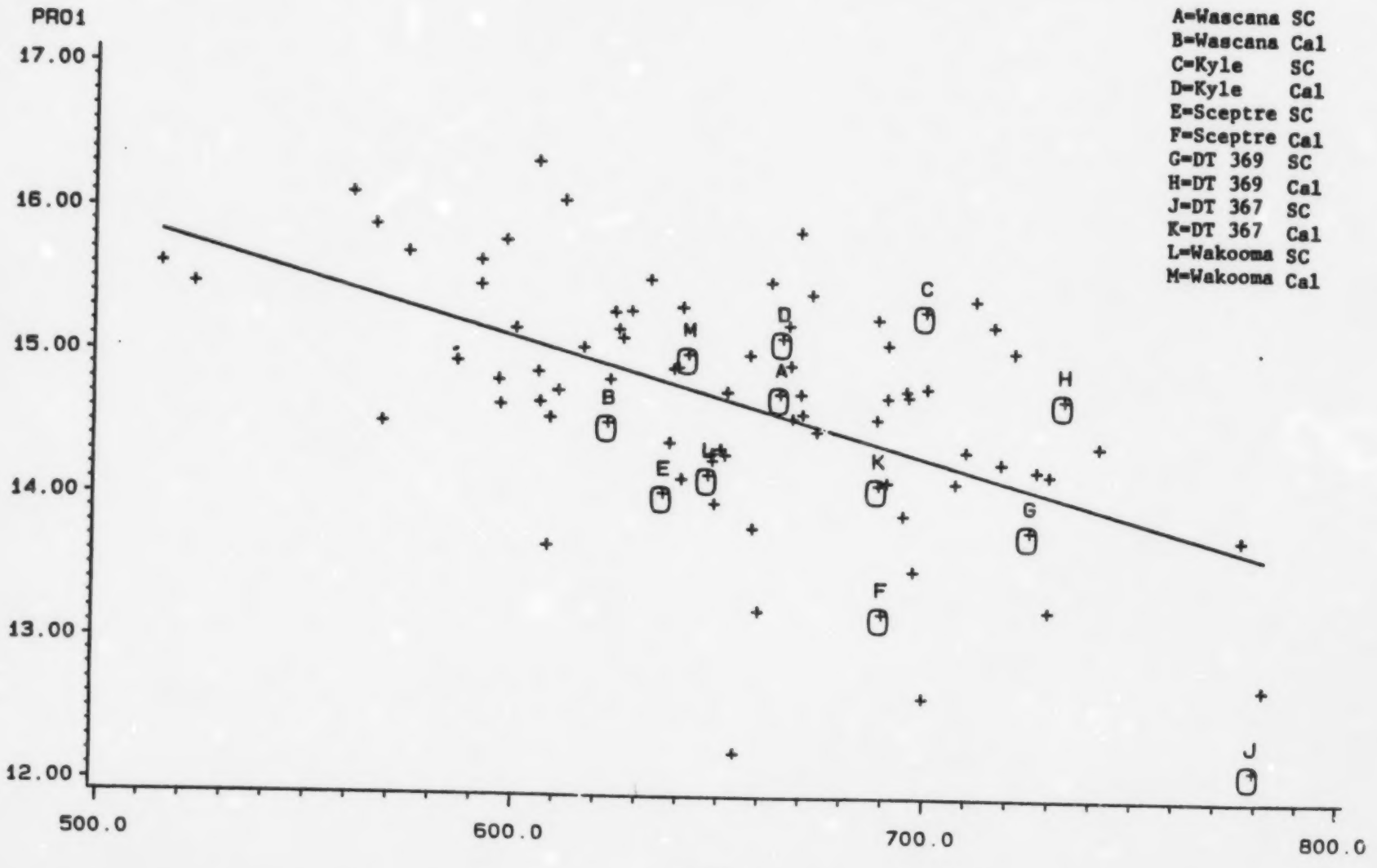


Figure 5. Regression of protein (%) on grain yield (gms/plot) (b= -0.0083**, r^2= 0.286).

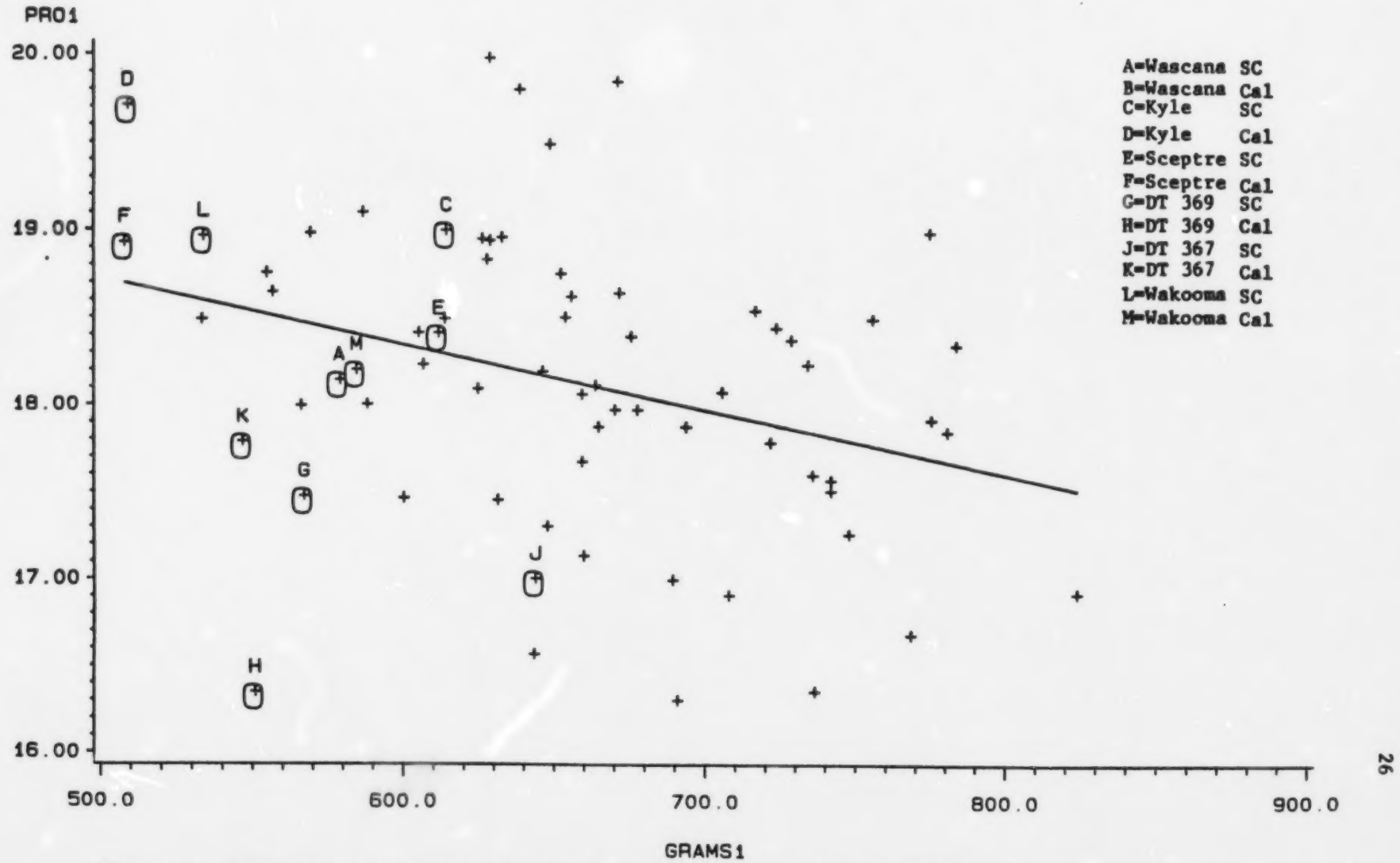


Figure 6. Regression of protein (%) on grain yield (gm/plot) ($b = -0.0037^{**}$, $r^2 = 0.104$).

CONCLUSIONS:

1. NIR technology can be routinely used in a Wheat breeding program to efficiently screen for protein content and kernel hardness.
2. NIR technology can be used to measure protein content in hard red spring, durum, and Canada Prairie Spring wheat.
3. For accurate protein measurements, separate equations must be used for hard and soft wheats.
4. For accurate protein and hardness measurements the grain should be equilibrated to constant moisture content prior to grinding. Following grinding, the grain should be stored in air-tight containers.
5. For all NIR analyses, the laboratory analyses used to calibrate the instrument must be very accurate.
6. For all NIR analyses, the instrument must be maintained in a constant temperature room.
7. Genetic variation exists for grain yield and protein.
8. There is a weak negative relationship between grain yield and protein.

REFERENCES

1. Williams, P.C., Norris, K.H. and Zarowski, W.S. 1982. Influence of temperature on estimation of protein and moisture in wheat by near-infrared reflectance. Cereal. Chem. 59(6):473-477.

2. Williams, P.C. 1979. Screening wheat for protein and hardness by near infrared reflectance spectroscopy. Cereal Chem. 56(3):169-172.

3. Amer. Assoc. of Cereal Chemists. Approved methods of th AACC. The Association: St. Paul, Minn. (1962).

4. Baker, R.J. 1977. Inheritance of kernel hardness in spring wheat. Crop Sci. 17:960-962.

Implications for the Saskatchewan Agriculture Industry

Scientists in Saskatchewan are now using NIR technology to more efficiently produce new wheat cultivars which combine high yields with desirable levels of kernel protein and hardness. NIR is an important tool to allow wheat breeders to respond more quickly to the demands of rapidly changing world markets. This means that Saskatchewan farmers will continue to offer high quality products matched to the end-use requirements of importing countries.

Recommendations for Producers or Extension Personnel

The conclusions from this work are most applicable to Researchers. However, extension personnel should certainly realize that scientists are using such techniques and interact with those scientists to ensure that the concerns of producers are being met by the research. Extension personnel and producers should also benefit by understanding some of the limitations of NIR technology that is presently being used in grain elevators. They should realize that the techniques are not perfect and that errors are possible if they not carefully applied.

APPENDIX 1.

FINAL REPORT FOR 1986/87 OF THE FIRST YEAR OF SASKATCHEWAN AGRICULTURE DEVELOPMENT FUND RESEARCH GRANT (previously referred to as SARF grant #1-85-22)

FINAL REPORT FOR 1986/87 OF THE FIRST YEAR OF BARF RESEARCH PROJECT
81-85-22

The Development of Techniques and Strategies of Selection for Grain Yield, Protein and Kernel Hardness in Wheat Using Near-Infrared Spectroscopy

PART I

1. Abstract

Near infrared (NIR) spectrophotometry has potential as an inexpensive and rapid means of measuring protein content and kernel hardness of wheat; this could significantly improve the efficiency of breeding programs. However, at present our knowledge of NIR is very limited. The objective of the present research project is to integrate such an instrument with a wheat breeding program. The results for the first year of this study are promising although most of the emphasis has been on collection of appropriate plant samples and development of methodology. Several plant populations have been developed from parents containing desirable quality attributes. Concerning instrumentation, it was found that to accurately measure kernel hardness using NIR, all samples should be at a similar moisture content prior to grinding; a chamber was therefore modified to equilibrate grain at 12-14 % moisture. A serious problem was discovered concerning the measurement of protein with the Neotec 51A NIR instrument; the instrument was found to be extremely susceptible to fluctuations of the ambient temperature. The NIR was therefore moved to a constant temperature room to overcome this problem. We are presently evaluating the reproducibility of the NIR to measure protein and kernel hardness following the methodology improvements outlined above.

2. Background and Objectives

In order to remain competitive on the world wheat market, Canada must develop wheats which match the quality requirements of the importing countries. For example, high protein wheats tend to yield less grain; therefore, it is poor economics to use such wheats to compete for markets that do not require high levels of protein. Two of the most important quality components of wheat are protein and kernel hardness. However, traditional methods of measuring these components are expensive, labour intensive and slow. Near Infrared (NIR) spectroscopy represents an attractive alternative to the traditional methods as it is rapid and inexpensive. In 1977, R.D. Rosenthal presented a paper at the Amer. Assoc. of Cereal Chemists meetings, discussing the potential of using NIR to measure many agriculturally important constituents (e.g. protein, moisture, starch, etc.). Dr. P.C. Williams has successfully measured protein (1,2), moisture (1), and hardness (2) with NIR. However, NIR still remains more of an art than a routine technique and considerable study is required to ensure that it can be readily adapted by breeding programs. However, the advantages of integrating NIR into Canadian wheat breeding would be considerable.

Great variation exists in the genetic wheat stocks for most quality characteristics. Researchers such as Kushnir and Halloran (Australia) have demonstrated that desirable grain characteristics, such as protein content and kernel weight, can be transferred from ancestral wheats into adapted

varieties.

The objective of this research proposal, in general terms, is to increase the efficiency of wheat breeding programs by utilizing NIR technology to measure quality components such as protein and kernel hardness. Specifically, we plan to:

(a) Develop and evaluate calibration constants for a near infrared reflectance spectrophotometer suitable for measuring protein content and kernel hardness in wheat.
(b) Formulate selection strategies to select for grain yield and protein content in both Hard Red Spring and Amber Durum Wheat classes; and grain yield, protein content and kernel hardness in the Canadian Prairie Spring Wheat class (HY320 type).
(c) Implement evaluation and selection strategies that increase the efficiency of developing improved cultivars of Canada Prairie Spring, Hard Red Spring and Amber Durum Wheat classes.

3. Experimental Methods:

(a) Plant samples

Canadian Western Amber Durum
Five Triticum diccocum lines which have high protein content but are unadapted to Saskatchewan and low yielding were hybridized previously with DT367, a high yielding line (20 % higher than Wascana and Wakooma) but low in protein content. In 1985, the populations from these crosses were grown at Swift Current and Regina and 53 F6 and F8 lines were selected for further testing. During the winter of 1985-86, 265 head rows were grown at Brawley, Calif. to inbreed and multiply seed stocks. In 1986, 127 of the F8 and F10 lines were grown at Swift Current and Regina. Seed samples were collected from each of these lines grown at two locations for two years and also in a winter nursery.

Canada Prairie Spring
Thirty nine populations were produced previously to achieve the objective of developing high-yielding, sprouting-resistant white seeded cultivars suitable for registering in this new wheat class. In 1985, 1695 hybrid lines were grown at Swift Current and Regina from these 39 populations. From these, 422 lines were selected with 5 head-rows being established for each selected line (422 x 5 =2110) for multiplication and inbreeding in the Brawley winter nursery. In 1986, 920 lines were selected from the winter nursery and evaluated for performance relative to the CPS goal at Swift Current and Regina. Seed samples were collected from each of these lines grown at two locations for two years and also in a winter nursery.

(b) Sample processing
An equilibration chamber is used to condition grain samples prior to grinding for NIR analyses. The chamber is maintained at 21 deg C and 50 % relative humidity. The samples are stored in envelopes with computer-generated identification labels on them; they are placed in this chamber for at least one week in order to adjust the moisture content of the grain to between 12 and 14%. Grain samples are then ground using a Udy cyclone grinder (Udy Corp., Colorado) fitted with

a constant feed adaptor. Only enough samples are removed from the equilibration chamber as can be conveniently ground within a 2 hour period to prevent changes in grain moisture content prior to grinding. Immediately following grinding the samples are placed in metal containers and sealed to prevent moisture changes.

(c) Near infrared
The NIR instrument used in a Neotec 51A (Neotec, Silver Springs, MD). This instument uses six rotating filters in order to measure absorption at 768 wavelengths. The equation presently being evaluated for protein is the one supplied by Neotec. For kernel hardness, a wavelength of approximately 1620 nanometer is being used (2). The entire spectra of certain sample sets is being stored on a microcomputer.

(d) Lab analyses
Protein is being measured at the Swift Current Research Station using the Kjeldahl procedure (3). Moisture contents are being measured gravimetrically following 24 hours drying at 105 deg C. Kernel hardness is measured at the Winnipeg Research Station; the method involves determination of grinding times (4).

All data is being analyzed with SAS and SASGRAPH (SAS Institute,Cary,NC) running on a VAX 750 minicomputer (Digital Equipment Corp.).

4. Results and Discussion:

When wheats of differing hardness are ground, the resulting grinds have differing particle size distributions. Since the NIR is very sensitive to the particle size of a sample, it is this property which allows the NIR to measure kernel hardness. However, results obtained early in this study indicated that in order to determine kernel hardness accurately by NIR, it was necessary that all grain samples be brought to the same moisture content prior to grinding. The reason for this was that grain moisture content also affects particle size distribution during grinding. Therefore, differing moisture contents at time of grinding could be interpreted as hardness differences. Since most of the NIR results reported in the literature used grain of approximately 12 to 14 % moisture, we decided to bring our grain to this moisture content also, to aid with comparison of results with other groups. According to the literature, the conditions required to adjust unground wheat to 12 to 14 % moisture are 21 deg C and 50% humidity. As we did not have a chamber available which could produce such conditions, one was modified by the addition of a dehumidifier. A study indicated that grain at high (>15 %) or low (<8%) moisture would fall within the 12-14 % range after only a few days in the chamber. Routinely, all samples now remain within the chamber for at least one week prior to grinding.

Several populations, involving several hundred grain samples, were measured for protein content during the fall and winter months of 1986 using the NIR. Although the NIR-protein and Kjeldahl-protein values from some of these tests were highly correlated (R2>0.9), results of other tests indicated considerable variation between the lab (Kjeldahl) and NIR protein. A detailed study of the results indicated that we had a problem

with reproducibility from day to day. Considerable time was spent trying to determine the source of this problem. A trip was made to Winnipeg to discuss some of our results with Dr. Phil Williams (Grain Research Laboratory), a recognized expert in the field of NIR. With Dr. Williams cooperation, and discussions with others using similar NIR equipment (at the Brandon Research Station), we established that the instrument was responding to variations of the ambient temperature (even though the specifications for the NIR claim that the instrument is stable to such temperature changes). Recently we were able to move the instrument to a constant temperature room and we are now in the process of evaluating results under these conditions. Dr. Williams is also providing us with monthly grain samples which are analysed for protein content by several labs to help in the calibration of the instrument.

5. Impact

The results from the first year of this study have demonstrated the importance of sample processing and methodology to achieve the accuracy demanded by wheat breeding programs. We have determined that samples should be equilibrated to 12-14 % moisture prior to grinding. We have also identified the need to maintain constant temperature operating conditions for the NIR instrument. These findings have increased the accuracy of measuring the target parameters of kernel hardness and grain protein content. This lays a better foundation upon which to proceed to develop reliable technology to select for these characteristics in a wheat cultivar improvement program

The long term impact of these 1986/87 results will contribute to the development of wheat cultivars better suited to meet the requirements of producers and to increase our competitive edge in international grain markets.

References

1. Williams, P.C., Norris, K.H. and Zarowski, W.S.1982. Influence of temperature on estimation of protein and moisture in wheat by near-infrared reflectance. Cereal. Chem. 59(6):473-477.

2. Williams, P.C. 1979. Screening wheat for protein and hardness by near infrared reflectance spectroscopy. Cereal Chem. 56(3):169-172.

3. Amer. Assoc. of Cereal Chemists. Approved methods of th AACC. The Association: St. Paul, Minn. (1962).

4. Baker, R.J. 1977. Inheritance of kernel hardness in spring wheat. Crop Sci. 17:960-962.

Financial Statement

As mentioned in a previous Progress report, there was considerable delay involved in signing this first year agreement which resulted in delays of hiring, purchasing and, therefore, results. We did not receive the unsigned contract until June 2, 1986 and then there were further delays with the Swift Current Agricultural and Exhibition Association (to whom we are indebted for the management of financial matters). The result was that we did not receive the first allotment until Sept. 1986. Hopefully, the first allotment for 1987 can be received by July 1987.

Following is the financial statement for the period April 1, 1986 to June 4, 1987:

Funding received from SARF grant			16,200.00
Final payment (contingent upon acceptable final report)			1,800.00
			18,000.00
Expenses:			
Materials and supplies		730.60	
Employee costs :	Salary	9,336.00	
	Employer's contribution	3,445.54	
Computer and statistical analyses		400.00	
Travel (trip to Winnipeg)		389.55	
Service charges		395.55	
		14,918.09	14,918.09
Anticipated expenditure to June 30, 1987:			
Employee costs:	Salary	1,291.08	
	Employer's contribution	1,100.00 (est.)	
		2,391.08	2,391.08
		Total expenses	17,309.17
Carryover as of June 30/87 (estimate)			690.83

Part II ABSTRACT

In the world wheat market, as in other markets, exporting countries must match the quality requirements of the importing countries, or sell their product at a discount. For example, it is poor economics to sell a high protein wheat into a market with a quality requirement for low protein wheat. However, presently it is very expensive, labour intensive and time-consuming for wheat breeders to screen all of their wheat lines for protein and kernel hardness. Near infrared instruments have the potential of measuring protein content and kernel hardness at a low cost. However, it involves fairly new and sophisticated technology, and our knowledge is still somewhat limited in this area. The main objective of this study is to attempt to adapt a near infrared instrument to a wheat breeding program in the hope of greatly improving the efficiency and decreasing the expense when breeding to improve the quality characteristics of wheat. Following the first year of this study much of the plant material has been obtained and much has been learned concerning the conditions required for plant processing and instrument operation.

APPENDIX 2.

FINAL REPORT FOR 1987/88 OF THE SECOND YEAR OF SASKATCHEWAN AGRICULTURE DEVELOPMENT FUND RESEARCH GRANT (previously referred to as SARF grant #1-85-22)

The Development of Techniques and Strategies of Selection for Grain Yield, Protein and Kernel Hardness in Wheat Using Near-Infrared Spectroscopy.

Abstract

Our results indicate that near infrared spectroscopy (NIR) can be used in a wheat breeding program to increase efficiency and lower costs required to screen for certain kernel quality characteristics. Kernel hardness and protein are two of the most important quality components of Canadian wheats and laboratory procedures for both are labour intensive and expensive. NIR determinations for kernel protein, hardness and moisture can be done simultaneously and with good accuracy. Complete analysis on a ground sample takes only 30 seconds. However, instrument calibration must be done carefully and sample processing procedures must be strictly followed.

Background and Objectives (unchanged from 1986/87 report)

In order to remain competitive on the world wheat market, Canada must develop wheats which match the quality requirements of the importing countries. For example, high protein wheats tend to yield less grain; therefore, it is poor economics to use such wheats to compete for markets that do not require high levels of protein. Two of the most important quality components of wheat are protein and kernel hardness. However, traditional methods of measuring these components are expensive, labour intensive and slow. Near Infrared (NIR) spectroscopy represents an attractive alternative to the traditional methods as it is rapid and inexpensive. In 1977, R.D. Rosenthal presented a paper at the Amer. Assoc. of Cereal Chemists meetings, discussing the potential of using NIR to measure many agriculturally important constituents (e.g. protein, moisture, starch, etc.). Dr. P.C. Williams has successfully measured protein (1,2), moisture (1), and hardness (2) with NIR. However, NIR still remains more of an art than a routine technique and considerable study is required to ensure that it can be readily adapted by breeding programs. However, the advantages of integrating NIR into Canadian wheat breeding would be considerable.

Great variation exists in the genetic wheat stocks for most quality characteristics. Researchers such as Kushnir and Halloran (Australia) have demonstrated that desirable grain characteristics, such as protein content and kernel weight, can be transferred from ancestral wheats into adapted varieties.

The objective of this research proposal, in general terms, is to increase the efficiency of wheat breeding programs by utilizing NIR technology to measure quality components such as protein and kernel hardness.

Specifically, we plan to:

(a) Develop and evaluate calibration constants for a near infrared reflectance spectrophotometer suitable for measuring protein content and kernel hardness in wheat.
(b) Formulate selection strategies to select for grain yield and protein content in both Hard Red Spring and Amber Durum Wheat classes; and grain yield, protein content and kernel hardness in the Canadian Prairie Spring

Wheat class (HY320 type).

(c) Implement evaluation and selection strategies that increase the efficiency of developing improved cultivars of Canada Prairie Spring, Hard Red Spring and Amber Durum Wheat classes.

During the first year of our research it was discovered that the instrument was extremely sensitive to fluctuations in ambient temperature (contrary to claims of the manufacturer). Therefore, a major objective for this period was to determine instrument accuracy and reproducibility after moving it to a constant

Experimental Methods:

(a) Plant samples

Canadian Western Amber Durum

Five Triticum diccocum lines which have high protein content but are unadapted to Saskatchewan, and low yielding, were hybridized previously with DT367, a high yielding line (20% higher than Wascana and Wakooma) but low in protein content. In 1985 the populations from these crosses were grown at Swift Current and Regina and 53 F_6 F_8 lines were selected for further testing. During the winter of 1985-86, 265 head rows were grown at Brawley, Calif. to inbreed and multiply seed stocks. In 1986, 127 of the F_8 and F_{10} lines were grown at Swift Current and Regina. In 1987, 63 lines with improved protein content and good agronomic characteristics were grown in replicated tests at up to five locations. Seed samples were collected from each of these lines for each year and ea

Canadian Prairie Spring

Thirty nine populations were produced previously to achieve the objective of developing high-yielding, sprouting-resistant, white-seeded cultivars suitable for registering in this new wheat class. In 1985, 1695 hybrid lines, from these 39 populations, were grown at Swift Current and Regina. From these, 422 lines were selected with 5 head-rows being established for each selected line (422 x 5 = 2110) for multiplication and inbreeding in the Brawley winter nursery. In 1986, 920 lines were selected from the winter nursery and evaluated for performance relative to the CPS goal at Swift Current and Regina. In 1987, 1316 lines were evaluated for performance against the white CPS goal at two locations. Seed samples were collected from each of these lines grown at two locations for two years and also in a winter nursery.

Canada Western Hard Red Spring

Three populations were produced previously to achieve the objective of developing a hard red spring wheat with elevated protein and/or grain yield. In 1986, 614 F_4 lines were evluated in replicated tests at two locations. In 1987, 349 F_6 lines were progeny tested for elevated yield and/or protein content at two locations. As was done for the other classes of wheat, grain samples were collected from each of these lines f We also received a set of wheat samples, which are used as protein standards, from Dr. P. Williams of the Grain Research Laboratory in Winnipeg. These were used in the calibration of the NIR. We are presently awaiting the arrival of calibration samples for kernel hardness.

(b) Sample processing

An equilibration chamber is used to condition grain samples prior to grinding for NIR analyses. The chamber is maintained at 21 deg C and 50 % relative humidity. The samples are stored in envelopes with

computer-generated identification labels on them; they are placed in this chamber for at least one week in order to adjust the moisture content of the grain to between 12 and 14%. Grain samples are then ground using a Udy cyclone grinder (Udy Corp., Colorado) fitted with a constant feed adaptor. Only enough samples are removed from the equilibration chamber as can be conveniently ground within a 2 hour period to prevent changes in grain moisture content prior to grinding. Immediately following grinding the samples are placed in metal containers and sealed to prevent moisture changes.

(c) Near infrared

The NIR instrument used in a Neotec 51A (Neotec, Silver Springs, MD). This instument uses six rotating filters in order to measure absorption at 768 wavelengths. The equation presently being evaluated for protein is the one supplied by Neotec. For kernel hardness, a wavelength of approximately 1620 nanometer is being used (2). The entire spectra of certain sample sets is being stored on a microcomputer.

(d) Lab analyses

Protein is being measured at the Swift Current Research Station using the Kjeldahl procedure (3). Moisture contents are being measured gravimetrically following 24 hours drying at 105 deg C. Kernel hardness is measured at the Winnipeg Research Station; the method involves determination of grinding times (4).

(e) Data analyses

All data are being analyzed with SAS and SASGRAPH (SAS Institute,Cary,NC) running on a Microvax 2000 (Digital Equipment Corp.).

Results and Discussion:

An immediate concern following the first year of research was the reproducibility of the measurements. Our results indicated that the reproducibilty problems which we had experienced were entirely corrected by moving the instrument to a constant temperature room.

The NIR instrument was supplied with a set of wavelengths and a math treatment for the prediction of protein in wheat. In order to test the accuracy of this equation we analyzed the set of samples sent to us by the GRL laboratory in Winnipeg. They had determined protein content of each of these samples at least 20 different times using the macro-Kjeldahl procedure (3). The results from the NIR correlated very highly with the true values for this set of samples (linear correlation coefficient > 0.95). Only minor corrections were required to our equation. This were very encouraging results.

The correlation was not as good, however, between NIR predicted protein values and Kjeldahl protein on samples collected from several field trials. Further study indicated that there were two reasons for this.

The first reason was that the laboratory procedure for the determination for protein (ie. Kjeldahl) was found to be more variable than was previously realized. Through contact with other research establishments we found that the accuracy of the Kjeldahl protein method was of concern to others also. This is very important because the NIR can only be as accurate as the laboratory method used to calibrate it.

The second reason that the correlation was not very good between NIR predicted protein values and Kjeldahl protein on samples collected from several field trials was found to relate to kernel hardness. The equation used by the NIR worked well for hard wheats, however, it overestimated (underestimated) protein of soft wheats by approximately 0.5%. The solution was simply to have two equations, one for hard and one for soft wheats.

The results concerning the protein determination are presently very encouraging. The NIR results correlated very highly with laboratory results for wheats from field trials when we (1) ensured that the the laboratory values for protein were highly accurate, and (2) separate equations were used for hard and soft wheats.
We have noticed some discrepancies between different NIR instruments in the results predicted for protein. We believe that this is certainly a possibility which may arise due to the manner in which the instruments are calibrated. For example, it is entirely possible that the protein values from one instrument may be dependent upon kernel hardness, moisture or some unknown characteristic while the values from the other instrument may be independent of these other characteristics. The reason is that NIR instruments from different manufacturers use different regions of the NIR spectrum and different math treatments to predict protein. This means that extreme care must be taken when calibrating an instrument or interpreting results from it.

Results indicate that the NIR can predict kernel hardness very accurately. However, to date we have only calibrated the instrument using grinding-times as the laboratory method. We are presently waiting for a very accurate set of hardness samples to arrive from GRL in Winnipeg.

Conclusions:

Kernel protein, hardness and moisture can be accurately determined using the Neotec 51A NIR instrument. However, the following precautions must be taken for the various constituents:

1. For all analyses the instrument must be maintained in a constant temperature room.
2. For all analyses the laboratory analyses used to calibrate the instrument must be very accurate.
3. For accurate protein and hardness measurements the grain should be equilibrated to constant moisture content prior to grinding. Following grinding, the grain should be stored in air-tight containers.
4. For accurate protein measurements separate equations must be used for hard and soft wheats.

These results indicate that NIR technology should dramatically improve the efficiency of wheat breeding programs concerned with attempting to develop wheats of specific protein and hardness characteristics.

References

1. Williams, P.C., Norris, K.H. and Zarowski, W.S.1982. Influence of temperature on estimation of protein and moisture in wheat by near-infrared reflectance. Cereal. Chem. 59(6):473-477.
2. Williams, P.C. 1979. Screening wheat for protein and hardness by near infrared reflectance spectroscopy. Cereal Chem. 56(3):169-172.
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Association: St. Paul, Minn. (1962).
4. Baker, R.J. 1977. Inheritance of kernel hardness in spring wheat. Crop Sci. 17:960-962.

Implications for the Saskatchewan Agriculture Industry

New applications of NIR technology to the human and livestock feed industries offer opportunities in efficiencies of production. Knowledge and expertise on the use of NIR that has been developed through this project will be available to Saskatchewan agricultural industries that wish to make use of it. Selecting for protein content and/or kernel hardness by use of NIR technology in the CWRS, CWAD, and CPS wheat classes has already started. Wheat cultivars selected for increased grain yield and/or protein content might be available within five or six years.

Brief report in lay language outlining specific practical recommendations for producers or extension personnel:

Some NIR instruments used to measure protein content are sensitive to temperature fluctuations, dust and operator. Producers who have protein measured on their grain samples should be cautious about accepting the results provided.

Financial Statement

Reporting for the first one-year contract terminated on July 20, 1987. Therefore, the financial statement for the second year of this contract commences July 21, 1987.

Period July 21, 1987 to Aug. 24, 1988:

Funding from SARF (to present):		$16,200.00
Final payment (contingent upon final report)		$ 1,800.00
Total		$18,000.00
Expenses:		
Deficit from previous year	$ 326.81	
Materials and supplies	$ 1,608.85	
Employee costs:		
Salary and employer contributions	$11,820.40	
Workers compensation	$ 180.05	
Service and administrative charges	$ 840.20	
	$14,776.31	
Forecast of expenses to Sept 16/88:		
Employee costs:		
Salary and employer contributions	$ 3,134.80	
Service and administrative charges	$ 90.00	
	$ 3,224.80	
Total expenses for period July 21/87-Sept 16/88		$18,001.11

APPENDIX 3.

Equations to predict protein in wheat on the Neotec 51A near-infrared spectrophotometer.

This instrument uses 6 rotating interference filters to provide measurements at 768 different wavelengths. The principle is that the effective center wavelength of the filter shifts to longer wavelengths as the filter is tilted relative to the light source. As the angle of the filter changes, so does the light path through the filter. This change in path length produces an interference pattern effect, such that a single wavelength is reinforced and passed, while all others are blocked. The measurements are taken at 768 different points (128 on each filter), called 'pulse points' (PP). For this discussion, it can be assumed that the pulse points simply represent different wavelengths, although the band width is not the same for all pulse points.

The math treatment recommended by Neotec for protein in wheat is known as Delta OD / Delta OD, or Math #5. Essentially this means that the slope, or rate of change (Delta) of Optical Density (OD) in one region of the spectrum is divided by the slope (or Delta OD) in another region. The specific regions of the spectrum which are selected are those that have been found to be highly correlated with protein. The math treatment which is used is also selected to minimize certain problems, such as non-uniform sample grinding.

To predict protein Neotec then uses the spectrum values at selected pulse points (or wavelengths) in a simple linear regression equation :

$$\% \text{ Protein} = C_0 + C_1(\text{Ratio})$$

C_0 = intercept = 32.8
C_1 = slope = 4773.8

$$\text{Ratio} = \frac{(\text{OD at PP 446} - \text{OD at PP 407})}{(\text{OD at PP 553} - \text{OD at PP 514})} \times \frac{1}{100}$$